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Type studies on *Amauroderma* species described by J.D. Zhao et al. and the phylogeny of species in China

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Abstract — Holotypes of eight *Amauroderma* species described by Ji-Ding Zhao and co-workers, together with 93 additional specimens collected from China, were examined. Four names are demoted to synonymy (*A. amoiense* and *A. wuzhishanense* = *A. rugosum; A. dayaoshanense* = *Pyrrhoderma sendaiense; A. fujianense* = *Ganoderma fornicatum*), two represent accepted species (*A. austrosinense*, *A. yunnanense*), and two are of uncertain application (*A. longgangense*, *A. jiangxiense*) because of the poor or sterile condition of the holotype material. Illustrated descriptions of *A. austrosinense* and *A. yunnanense* are given based on the holotypes. Four other species reported from China (*A. perplexum*, *A. rugosum*, *A. subresinosum*, *A. yunnanense*) were sequenced for molecular analysis, and a phylogenetic tree was constructed by maximum parsimony and Bayesian analyses of the nuclear internal transcribed spacer of ribosomal DNA (ITS rDNA) sequences. A key to accepted *Amauroderma* species in China is provided.

Key words — *Ganodermataceae*, morphology, phylogenetics, taxonomy

Introduction

Amauroderma Murrill was established by Murrill and typified by *A. regulicolor* (Berk. ex Cooke) Murrill (Murrill 1905). This genus is mainly characterized by sessile or stipitate (mesopodal, pleuropodal) basidiocarps with a laccate or dull pileus; a trimitic hyphal system; and ellipsoid, subglobose to globose bitunicate basidiospores with an asperulate inner wall (Furtado 1981, Ryvarden 2004). Macroscopically, *Amauroderma* shares with *Ganoderma* P. Karst. a similar basidiocarp shape of central or lateral stipe and a laccate or dull surface. *Ganoderma* can be separated from *Amauroderma* by its distinctly truncate basidiospores, and most *Ganoderma* species grow on dead wood, while most *Amauroderma* species grow in the ground from buried roots (Ryvarden 2004).

Amauroderma is widely distributed in the tropics and subtropics south of 25°N in China within Guangdong, Guangxi, Yunnan, and Hainan Provinces. *Amauroderma* species are regarded as economically valuable because of their famous medicinal properties and pathogenicity (Dai et al. 2007, 2009; Jiao et al. 2013; Chan et al. 2013). Index Fungorum (www.indexfungorum.org) has listed 126 names under this genus. In China, taxonomic research on *Amauroderma* began with Teng, who recorded 10 species (Teng 1936, 1939, 1963). Later Zhao et al. (1979, 1983, 1984) and Zhao & Zhang (1986, 1987a,b, 2000) reported 20 species, nine of which were new. The types of these new species [except *Amauroderma guangxiense* J.D. Zhao & X.Q. Zhang (not traced)] are preserved in the Herbarium Mycologicum Academiae Sinicae (HMAS), the Institute of Microbiology, Chinese Academy of Sciences. Tai (1979) and Fan & Liu (1990) have also contributed to *Amauroderma* records in China.

In this study we investigated the type specimens of *Amauroderma* described by Ji-Ding Zhao and his colleagues and 93 other recently collected specimens from southern China based on morphological characters and rDNA sequence analyses. The taxa are presented below in alphabetical order according to the original names.

Materials and Methods

Morphology

The studied specimens were deposited in the Herbaria of the Institute of Applied Ecology, Chinese Academy of Sciences (IFP); the Institute of Microbiology, Chinese Academy of Sciences (HMAS); and the Institute of Microbiology, Beijing Forestry University (BJFC). Microscopic procedures followed He & Dai (2012). Sections were studied at magnifications up to 1000× using a Nikon Eclipse 80i microscope with phase contrast illumination. Measurements were made from sections stained with Cotton Blue. In presenting basidiospore size variation, 5% of measurements are excluded from each end of the range and given in parentheses. The following abbreviations are used: CB = Cotton Blue, CB+ = cyanophilous, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = negative in Melzer's reagent, KOH = 5% potassium hydroxide, L = mean basidiospore length (arithmetic average of all basidiospores), W = mean basidiospore width (arithmetic average of all basidiospores), Q = variation in the L/W ratios between the specimens studied, and n = number of basidiospores measured from given number of specimens. Special color terms follow Petersen (1996).

Molecular phylogeny

Amplification of ITS sequences from herbarium specimens was conducted using Phire® Plant Direct PCR Kit (Finnzymes, Finland) with ITS5 and ITS4 primers (White et al. 1990). PCR procedure was as follows: initial denaturation at 98 °C for 5 min, followed by 39 cycles at 98 °C for 5 s, 59 °C for 10 s and 72 °C for 5 s, and a final extension at 72 °C for 2 min. PCR amplification was confirmed on 1% agarose electrophoresis gels

TABLE 1. ITS sequences of *Amauroderma* and *Tomophagus* species used in the molecular analyses.

SPECIES	VOUCHER	LOCALITY	GENBANK NO.
A. perplexum	Cui 6496	Hainan, China	KJ531650
	Dai 10811	Hainan, China	KJ531651
	Wei 5562	Hainan, China	KJ531652
A. rugosum	Cui 6285	Hainan, China	KJ531656
	Zhou 153	Guangxi, China	KJ531657
	Dai 7862	Hainan, China	KJ531658
	Zhou 136	Guangxi, China	KJ531659
	Zhou 347	Guangxi, China	KJ531660
	Zhou 66	Guangxi, China	KJ531661
	Yuan 4554	Hainan, China	KJ531662
	Dai 9904	Hainan, China	KJ531663
	Cui 9011	Guangdong, China	KJ531664
	Cui 9012	Guangdong, China	KJ531665
	Wei 5234	Hainan, China	KJ531666
	Cui 8882	Guangdong, China	KJ531667
	Dai 9553	Hainan, China	KJ531668
	Dai 9566	Hainan, China	KJ531669
	Dai 4345	Hainan, China	KJ531670
	Dai 10040	Hainan, China	KJ531671
	Cui 4078	Fujian, China	KJ531672
	Dai 10307	Hainan, China	KJ531673
	Zhou 523	Guangxi, China	KJ531674
	Zhou 547	Guangxi, China	KJ531675
	Dai 12324	Yunnan, China	KJ531676
	Dai 12390	Yunnan, China	KJ531677
	Cui 9006	Guangdong, China	KJ531678
	THP 30	Unknown	HM 480835
A. subresinosum	Wei 5569	Hainan, China	KJ531649
	ML 288	Malaysia	JQ409358
A. yunnanense	Cui 7974	Yunnan, China	KJ531653
	Dai 13021	Yunnan, China	KJ531654
	Yuan 2253	Yunnan, China	KJ531655
T. colossus	CGMCC 5.763	Philippines	JQ081068

stained with ethidium bromide (Stöger et al. 2006). The PCR products were purified and directly sequenced in Beijing Genomics Institute, China, with the same primers. The most similar sequences (TABLE 1) were retrieved from GenBank (http://www.ncbi.nlm.gov) using the BLAST option. Sequences were aligned using ClustalX (Thompson et al.

1997). Alignment was manually adjusted to allow maximum alignment and minimize gaps, and deposited in TreeBASE (http://treebase.org/treebase-web/; submission ID: 17050). Identity/similarity between two sequences was calculated using the "pairwise alignment, calculation of the similarity/identity" option of BioEdit v. 7.0.5 (Hall 2005). Maximum parsimony and Bayesian analysis were applied to the ITS dataset. All characters were weighted and gaps were treated as missing data. Maximum parsimony analysis (PAUP* version 4.0b10) was used (Swofford 2002). Trees were inferred using the heuristic search option with TBR branch swapping and 1,000 random sequence additions. Max-trees were set to auto-increase, branches of zero length were collapsed, and all parsimonious trees were saved. Clade stability was assessed using a bootstrap (BT) analysis with 1,000 replicates (Felsenstein 1985). Descriptive tree statistics, including tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for all trees generated under different optimality criteria.

JModelTest (Posada 2008) was used to determine the best-fit evolution model for each data set for Bayesian inference (BY). Bayesian inference was calculated with MrBayes3.1.2 with a general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck 2003). Four simultaneous Markov chains were run with 3 million generations, and trees were sampled every 100 generations. Trees generated for the first one-fourth generations were discarded as burn-in. A 50% majority rule consensus tree of all remaining trees was calculated. Branches that received maximum parsimony bootstrap support (BSS) and Bayesian posterior probabilities (BPP) respectively greater than or equal to 75% and 0.95 were considered as significantly supported.

Taxonomy

We studied eight species of *Amauroderma* (*Ganodermataceae*) described from China by Ji-Ding Zhao et al. Four of the eight names are synonymized, two are accepted, and two are of uncertain application.

Amauroderma amoiense J.D. Zhao & L.W. Hsu, Acta Mycol. Sinica 2: 164. 1983.

HOLOTYPE: China. Fujian Prov., Xiamen, sandy land, 13.VI.1976, Li Hui-zhong & Zhang Xiao-qing 28 (HMAS 42784).

= ***Amauroderma rugosum*** (Blume & T. Nees) Torrend, Brotéria, Sér. Bot. 18: 127. 1920.

The type material is sterile, but all other characters fit *A. rugosum* well. For a detailed description of *A. rugosum*, see Ryvarden & Johansen (1980).

Amauroderma austrosinense J.D. Zhao & L.W. Hsu, Acta Mycol. Sinica 3: 20. 1984. FIG 1

HOLOTYPE: China. Hainan Prov., Bawangling, ground, 20.IV.1977, Han Shu-jin 902 (HMAS 42695).

BASIDIOCARPS annual, stipitate, umbelliform. PILEUS single, suborbicular to circular, glabrous, dull, clay-buff to fulvous brown, densely concentrically zoned

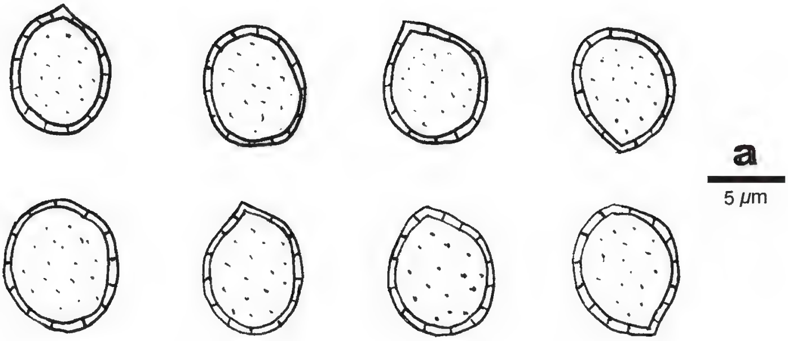

Fig. 1. *Amauroderma austrosinense* (holotype): basidiospores.

and slightly irregularly radially furrowed; MARGIN obtuse and deflexed when dry; STIPE sub-central or eccentric, concolorous with pileus, cavitate, up to 12 cm long and 1 cm thick. CONTEXT cinnamon, dense and hard, homogenous, up to 4 mm thick. PORE SURFACE cream white when fresh, turning brown with age; PORES circular, entire, 6–8 per mm. TUBES woody, up to 3 mm long.

HYPHAL SYSTEM trimitic; GENERATIVE HYPHAE with clamp connections, hyaline, thin-walled, 2.8–4 µm in diam; SKELETAL HYPHAE pale yellow to almost colorless, sparsely branched, 3.7–5.2 µm in diam; BINDING HYPHAE hyaline, branched, tortuous, 1.5–2.2 µm in diam; all the hyphae IKI–, CB+; tissues darkening in KOH. BASIDIOSPORES subglobose to globose, faintly yellowish, IKI–, CB+, doubled-walled, exospore smooth, endospore with conspicuous echinule, (6.5–)6.8–7.6(–8) × (5.9–)6.2–7(–7.3) µm, L = 7.34 µm, W = 6.58 µm, Q = 1.12 (n = 30/1).

ADDITIONAL SPECIMEN EXAMINED: **CHINA, HAINAN PROV.**, Changjiang County, Bawangling Nat. Res., ground, 20.IV.1977 (HMAS 42695).

REMARKS: *Amauroderma austrosinense* is an accepted species, remarkable for its umbelliform basidiocarps with distinct concentric rings, small pores, and basidiospores. *A. camerarium* (Berk.) J.S. Furtado is similar to *A. austrosinense* by sharing a clay-colored, densely concentrically zoned, and radially furrowed pileus. However, *A. camerarium* differs in its larger basidiospores (12–15 × 10–13 µm; Ryvarden 2004).

Amauroderma leucosporum Corner may be confused with *A. austrosinense* by having similar-sized subglobose to globose basidiospores and also not turning blood red on bruising, but *A. leucosporum* can be distinguished by its finely to distinctly villous pileus and stipe (Corner 1983).

Amauroderma dayaoshanense J.D. Zhao & X.Q. Zhang, Acta Mycol. Sinica 6: 5. 1987.

HOLOTYPE: China. Guangxi Auto. Reg., Dayaoshan, dead wood, 1982, Wei Bing-gang 70 (HMAS 48282).

= ***Pyrrhoderma sendaiense*** (Yasuda) Imazeki, Trans. Mycol. Soc. Japan 7: 4. 1966.

The type is sterile but has the characteristics of *P. sendaiense* (see Núñez & Ryvarden (2000) for a detailed description).

Amauroderma fujianense J.D. Zhao, L.W. Hsu & X.Q. Zhang, Acta Microbiol. Sinica 19: 275. 1979.

HOLOTYPE: China. Fujian Prov., Sanming County, rotten wood, 28.VI.1976, Zhang Xiao-qing 68 (HMAS 37919).

= ***Ganoderma fornicatum*** (Fr.) Pat., Bull. Soc. Mycol. Fr. 5: 71. 1889.

The holotype is sterile but represents a juvenile specimen of *G. fornicatum* (see Patouillard (1889) for a detailed description).

Amauroderma jiangxiense J.D. Zhao & X.Q. Zhang, Acta Mycol. Sinica 6: 206. 1987.

HOLOTYPE: China. Jiangxi Prov., Ji'an County, rotten wood, 15.III.1983, Yang Ren-gen 152 (HMAS 50418).

The basidiocarp is umbelliform and the pileus is black, laccate, and shiny, but the specimen is completely sterile. It probably represents a *Ganoderma* species, but the application of the name is uncertain.

Amauroderma longgangense J.D. Zhao & X.Q. Zhang, Acta Mycol. Sinica 5: 222. 1986.

HOLOTYPE: China. Guangxi Auto. Reg., Nonggang, on rotten wood, 1979, Wei Bing-gang 790111-G8 (HMAS 47621).

Basidiospores subglobose, 6–8.5 × 5–8 µm, with spinules 0.8–1.2 µm on sporoderm. However, the single-walled basidiospore obviously differs from the double-walled spore of other *Amauroderma* species. The application of the name is uncertain.

Amauroderma wuzhishanense J.D. Zhao & X.Q. Zhang, Acta Mycol. Sinica 6: 208. 1987.

HOLOTYPE: China. Hainan Prov., Wuzhishan, on rotten wood, 22.VII.1956, Jiang Guang-zheng (HMAS 19311).

= ***Amauroderma rugosum*** (Blume & T. Nees) Torrend, Brotéria, Sér. Bot. 18: 127. 1920.

The holotype is in accordance with *Amauroderma rugosum*, with its pileus obscurely zonate, radially quite rugose, and warted. For a detailed description of *A. rugosum*, see Ryvarden & Johansen (1980).

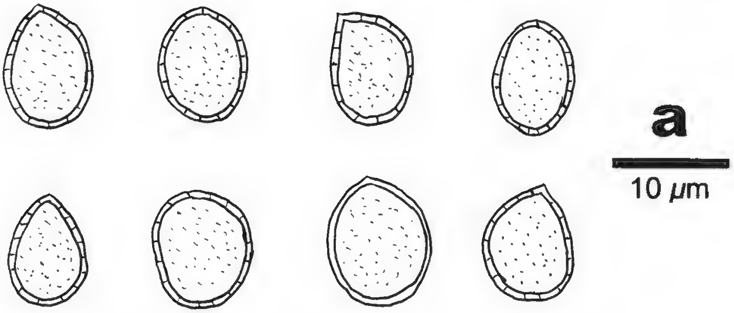

FIG. 2. *Amauroderma yunnanense* (holotype): basidiospores.

Amauroderma yunnanense J.D. Zhao & X.Q. Zhang, Acta Mycol. Sinica, Suppl. 1: 268. 1987 ["1986"]. FIG 2

HOLOTYPE: China. Yunnan Prov., Xichou County, ground, Xiaoqiaogou, 14.V.1959, Wang Qing-zhi 89 (HMAS 48231).

BASIDIOCARPS annual, centrally to laterally stipitate, coriaceous to corky, brittle when dry. PILEUS single, flabelliform, suborbicular, umbilicate, PILEAL SURFACE pale yellowish brown to dark brown, adpressed velutinate, with distinct concentric zones at the margin and fine furrows in the middle; MARGIN inflexed, acute or subacute, entire or slightly lacerated and thin; STIPE concolorous with the pileus, up to 7 cm long, inflated at base. CONTEXT homogeneous, white to pale yellow. PORE SURFACE white when fresh, pale straw yellow when dry, bruising unchanged; PORES angular, 2–3 per mm; TUBES concolorous with pore surface, up to 5 mm thick.

HYPHAL SYSTEM trimitic; GENERATIVE HYPHAE with clamps, hyaline, thin-walled, 2–4.3 µm in diam; SKELETAL HYPHAE thick-walled, pale yellow, arboriform, 3.5–5.5 µm in diam; BINDING HYPHAE hyaline, branched, tortuous, 1.8–2.5 µm in diam; all the hyphae IKI–, CB+; tissues darkening in KOH. BASIDIOSPORES broadly ellipsoid to subglobose, pale yellow brown, IKI–, CB+, doubled-walled, exospore smooth, hyaline, endospore with conspicuous echinule, (8.3–)8.9–10.3(–10.7) × (6.3–)6.7–8(–8.8) µm, L = 9.35 µm, W = 7.32 µm, Q = 1.22–1.34 (n = 120/4).

ADDITIONAL SPECIMENS EXAMINED: CHINA, YUNNAN PROV., Chuxiong, Zixishan Forest Park, ground, 8.IX.2006, Yuan 2253 (IFP 013207); Ailaoshan, 5.IX.2007, Yuan 3367 (IFP 013182); Kunming, Qiongzhusi Park, 21.X.2009, Cui 7974 (BJFC 006463); Xiaoshao Village, 22.IX.2012, Dai 13021 (IFP 019123).

REMARKS: *Amauroderma yunnanense* is an accepted species characterized by a brown tomentose pileus and stipe.

Amauroderma perplexum Corner and *A. yunnanense* both have a velutinate pileus and stipe as well as stipes that dilate at the base; *A. perplexum* can be separated by larger basidiospores, longer tubes, and a pore surface that changes color when bruised (Corner 1983).

Amauroderma partitum (Berk.) Wakef., which may be confused with *A. yunnanense* as they share similar angular pores that are 2–3 per mm, can be distinguished by its glabrous, slightly shiny pileus and larger basidiospores (12–15 × 8–10 µm; Ryvarden 2004).

Molecular phylogeny

The nuclear ITS rDNA dataset contains 32 sequences representing *A. perplexum*, *A. rugosum*, *A. subresinosum* (Murrill) Corner, and *A. yunnanense*, with *Tomophagus colossus* (Fr.) Murrill as outgroup (Table 1). After the ambiguous sites at both ends and the 5.8S region were trimmed off, the alignment comprised 561 characters, of which 462 were constant, 22 were variable but parsimony-uninformative, and 77 were parsimony-informative. Maximum parsimony analysis yielded 6 equally parsimonious trees (CI = 0.918, RI = 0.952, RC = 0.874, HI = 0.082). A strict consensus tree of these trees is presented in Figure 3. The 50% majority consensus tree generated by the Bayesian analysis showed a similar topology with the strict consensus MP tree, and only the topology from MP analysis is presented, while both bootstrap support value (BSS) and Bayesian posterior probabilities (BPP) are shown at the nodes (Fig. 3).

In the phylogenetic tree, each sampled *Amauroderma* species was resolved as a strongly supported clade (100% BSS and 1.00 BPP). Identity/similarity calculation result shows that identities of *Amauroderma* sequences are between 0.98 and 1. We failed to obtain sequences from the type specimens of *Amauroderma* described by Ji-Ding Zhao from China as the materials were too old to extract integrated DNA.

Key to species of *Amauroderma* in China

1. Pileal surface appressed velutinate 2
1. Pileal surface glabrous 3

2. Pores 2–3 per mm, pore surface straw yellow, not reddening on bruising,
spores (8.3–)8.9–10.3(–10.7) × (6.3–)6.7–8(–8.8) µm........... ***A. yunnanense***
2. Pores 4–6 per mm, pore surface cream white, reddening on bruising,
spores (10.3–)10.8–14(–14.8) × (8.9–)9.2–12(–13.1) µm......... ***A. perplexum***

3. Pileus surface laccate, spores ovoid to broadly ellipsoid,
(13.7–)14.6–17.1(–17.5) × (8.3–)8.9–10.2(–11.3) µm.......... ***A. subresinosum***
3. Pileal surface dull 4

4. Pileal clay-buff to fulvous brown, pores 7–8 per mm,
spores (6.5–)6.8–7.6(–8) × (5.9–)6.2–7(–7.3) µm .... ***A. austrosinense***
4. Pileus taupe, dark brown to black, pores 4–6 per mm,
spores (8.1–)8.4–11.7 (–12) × (7–)7.5–9.6(–10.3) µm.............. ***A. rugosum***

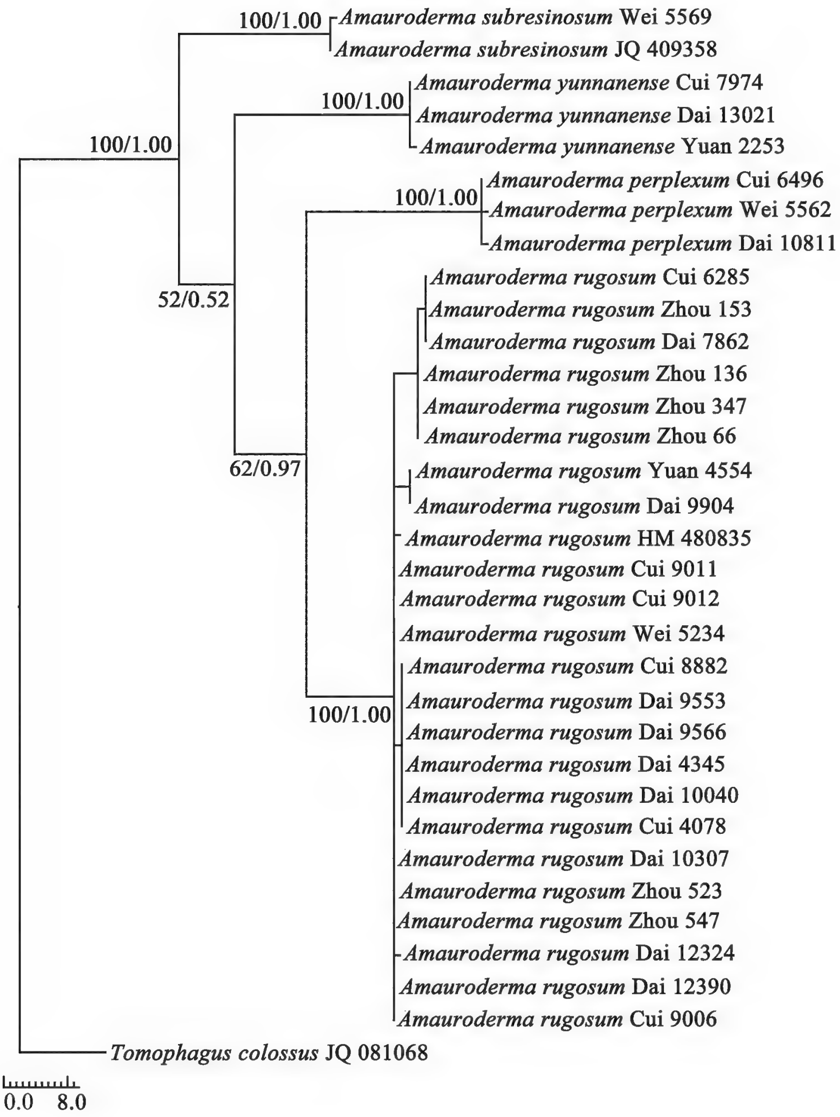
100/1.00
Amauroderma subresinosum Wei 5569
Amauroderma subresinosum JQ 409358
100/1.00
Amauroderma yunnanense Cui 7974
Amauroderma yunnanense Dai 13021
Amauroderma yunnanense Yuan 2253
100/1.00
100/1.00
Amauroderma perplexum Cui 6496
Amauroderma perplexum Wei 5562
Amauroderma perplexum Dai 10811
52/0.52
Amauroderma rugosum Cui 6285
Amauroderma rugosum Zhou 153
Amauroderma rugosum Dai 7862
Amauroderma rugosum Zhou 136
Amauroderma rugosum Zhou 347
Amauroderma rugosum Zhou 66
62/0.97
Amauroderma rugosum Yuan 4554
Amauroderma rugosum Dai 9904
Amauroderma rugosum HM 480835
Amauroderma rugosum Cui 9011
Amauroderma rugosum Cui 9012
Amauroderma rugosum Wei 5234
Amauroderma rugosum Cui 8882
Amauroderma rugosum Dai 9553
100/1.00
Amauroderma rugosum Dai 9566
Amauroderma rugosum Dai 4345
Amauroderma rugosum Dai 10040
Amauroderma rugosum Cui 4078
Amauroderma rugosum Dai 10307
Amauroderma rugosum Zhou 523
Amauroderma rugosum Zhou 547
Amauroderma rugosum Dai 12324
Amauroderma rugosum Dai 12390
Amauroderma rugosum Cui 9006
Tomophagus colossus JQ 081068
0.0
8.0

FIG. 3. Strict consensus tree obtained from Maximum Parsimony and Bayesian analyses of ITS sequences of *Amauroderma*. *Tomophagus colossus* was used as outgroup. Numbers at branches indicate parsimony bootstrap values and Bayesian posterior probabilities values higher than 50%.

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